Rejections under 35 U.S.C. §112, second paragraph

The claims have been rejected under 35 U.S.C. §112 as indefinite. These rejections are respectfully traversed if applied to the amended claims.

Claim 4 has been amended to define the tissue factor in terms of a proper Markush group.

Claim 6 has been amended to define the number of amino acids which can be substituted, deleted, or inserted as between one and ten amino acids. Support for the amendment is found on page 13, lines 28-31.

Claim 8 has been amended to delete the phrase "about".

Claim 20 has been amended to define the soluble tissue factor as having the sequence in Figure 2 from one to less than the full length protein of 263 amino acids.

Claim 22 has been amended to delete "essentially".

Claim 23 has been amended to define the tissue factor as having the amino acid sequence beginning from amino acid residue one, rather than the first amino acids. It should be noted, however, that it is well known that tissue factor can have a staggered N-terminus, since the cleavage site for the signal peptide is cleaved by a peptide which can also cleave immediately after the second amino acid of the mature peptide, in which case

the tissue factor would begin at amino acid three, rather than amino acid one.

Rejections under 35 U.S.C. §112, first paragraph

Claims 4-6, 8 and 21-26 have been rejected under 35 U.S.C. §112 on the basis that the claims are not enabled.

Claim 4, is directed to a tissue factor protein selected from the group consisting of:

tissue factor wherein a hydrophilic residue is substituted for a hydrophobic residue,

tissue factor wherein a cysteine or proline is substituted with any other amino acid residue,

tissue factor wherein a residue having an electropositive side chain is substituted for an electronegative residue, and

tissue factor wherein a residue having a bulky side chain is substituted for one not having a side chain.

The publication by Guha, et al., Proc. Natl. Acad. Sci. USA 83:299-302 (1986), reported the N-terminal amino acid sequence of a contaminant in the tissue factor protein which has subsequently been determined to be the complement inhibitor, CD59. The program "FASTA" was used to search a database that consists of 37,357,319 residues in 152,000 protein sequences, in which the human, bovine, mouse and rabbit tissue factor sequences are included. The programs shows the best match to be CD59 (71.43% similarity); no

match to any tissue factor was found within the 50 best matches, and the sequence similarity to human tissue factor is only 17.86%, the same as the random homology between unrelated sequences. The nucleotide sequence of CD59 was published by Okada, et al., Biochem. Biophys. Res. Comm. 162, 1553-1559 (1989), a copy of which is enclosed.

As demonstrated by "Tissue factor regulation and gene organization" by J.H. Morrissey, et al., Oxford Surveys on Eukaryotic Genes, ed. Norman Maclean, Vol. 6 (Oxford University Press 1989), pp. 67-84, especially pp. 76-77, there are naturally occurring variants in tissue factor that have no functional effects. There are differences between tissue factor from various species, for example, there is 70.4%, 57.2%, and 74.1% homology (i.e., sequence identity) between bovine and human, mouse, and rabbit tissue factors, respectively (Takayenoki, et al., Biochem. Biophys. Res. Commun. 181(3), 1145-1150 (1991). However, one can, and indeed did, use the sequence from human to clone the bovine TF.

More importantly, though, is that the claims are limited to a human tissue factor. The only tissue factor claimed is one having activity in promoting coagulation of human blood.

Applicants have indeed shown that a number of deletions can be made and activity retained. The entire transmembrane region can be deleted and the tissue factor retains activity. The first two

amino acids can be deleted, as occurs in nature, and there is no difference in activity. A tissue factor as small as 209 amino acids as subsequently been reported to be active in a factor VII assay.

In summary, applicants claims are limited to changes of ten or fewer specific substitutions having defined, well characterized effects. The literature supports applicants' statements that these modified human tissue factors would have activity.

With respect to glycosylation, the Examiner's attention is drawn to the specification at pages 32 to 43 of the published PCT application WO88/09817 by Mt. Sinai Medical Center and Yale University, which also discloses cloning and expression of human tissue factor. The tissue factor was expressed in *E. coli*, which does not glycosylate proteins, and demonstrated to have clotting activity.

Rejections under 35 U.S.C. §102(a)

Claims 20 and 21 were rejected under 35 U.S.C. §102(a) as disclosed by Abstract No. 1632 by Morrissey, et al. This rejection is respectfully traversed.

Morrissey, et al., reports on the isolation and characterization of full length human tissue facture, using the method of Guha, et al. (i.e., a factor VII affinity chromatography column).

As amended, claim 20 is directed to a soluble isolated tissue factor with the amino acid sequence shown in Figure 2 from amino acid one to less than amino acid 263, which is clearly different from, and could not be predicted from, the full length solubilized native human tissue factor. The non-glycosylated form was prepared by N-glycanase digestion of the full length protein.

Rejections under 35 U.S.C. §102(b)

Claims 4, 6 and 20 were rejected under 35 U.S.C. §102(b) as disclosed by Guha, et al., Proc. Natl. Acad. Sci. USA 83:299-302 (1986). This rejection is respectfully traversed.

As noted above, Guha, et al., reports the purification of a tissue factor including contaminants that were present in sufficient quantity that, when the mixture was sequence, the obtained sequence was that of a totally unrelated protein, a complement inhibitor protein now known as CD59. There is no disclosure of a **modified** tissue factor, the subject of claims 4, 6, and 20. Therefore, Guha, et al., does not anticipate claims 4, 6, and 20.

U.S. Patent No. 5,110,730; priority date of March 31, 1987

The Examiner's attention is drawn to the priority date of the present application: **February 12, 1987.** Accordingly, the Edgington, et al., patent is **not** prior art to the present

application, although the applications were co-pending and did claim the same subject matter.

It should be noted that claims to the tissue factor in a divisional of the application now issued have been allowed and the Patent Administrative Law Judge in the interference relating to the nucleic acid sequence encoding tissue factor, now pending between Edgington, et al., and Nemerson, et al., has requested that this be brought to the attention of the Examiners in all related applications.

Allowance of all claims 4-6, 8, and 20-26, as amended, is earnestly solicited.

Respectfully submitted,

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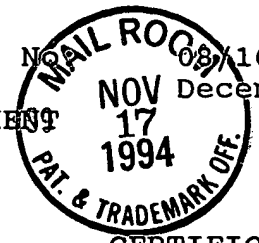
AMENDMENT



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I hereby certify that this paper, along with any paper referred to as being attached or enclosed, is being deposited with the United States Postal Service on the date shown below with sufficient postage as first-class mail in an envelope addressed to the Commissioner of Patents and Trademarks, Washington, D.C. 20231.

Date: November 14, 1994

Patricia Hilger